MICROEMULSIONS CONTAINING *Centella asiatica* EXTRACT AS ANTIOXIDANT AND ANTI-ACETYLCHOLINESTERASE AGENT

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DEDICATION

In the name of Allah, Most Gacious, Most merciful Muhammad S.A.W

This thesis is dedicated to

My beloved father
Haji Ghazali bin Haji Abdul Rahman

My beloved mother
Hajah Hanan binti Haji Bakar

My beloved wife
Nur Afni Syafinaz binti Budin

My supportive supervisor
Dr. Faridah binti Kormin

Thank you for everything

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ABSTRACT

The treatment of Alzheimer's disease (AD) has gained much attention due to the current and predicted prevalence of the disease. *Centella asiatica* hydroalcoholic extract (CAHE), lemongrass essential oil (LGEO) and virgin coconut oil (VCO) presenting antioxidant and acetylcholinesterase inhibition activity derived from natural products to enhanced memory of AD patients. In the present study, the ternary phase behaviour diagrams were constructed based on microemulsion (ME) containing CAHE with varying ratios of LGEO or VCO, and non-ionic surfactant, Tween 20 (T20) or Tween 80 (T80). The formulated MEs were characterized for the identification of functional groups by fourier transform infrared (FTIR), thermal behaviour by differential scanning colorimetry (DSC), and morphology and particle size by atomic force microscopy (AFM). The ME formulations were also tested for antioxidant activity by DPPH, ABTS, and FRAP assays, anti-acetylcholinesterase activity, *in-vitro* drug release, and cytotoxicity assay. Four ternary phase diagrams were successfully constructed and based on each ternary diagram, four transparent MEs were selected. The results of this study show that the particles of MEs were spherical in shape with an average particle size range of 0.1 – 0.2 µm. Thermodynamic and long-term storage stability tests conducted at room temperature showed that all the ME formulations had good stability without any phase separation. All the ME formulations revealed good antioxidant and anti-AChE activities. F1 (Tween 20/LGEO:CAHE) showed the highest antioxidant activities of DPPH, ABTS and FRAP is 79.3 %, 93 %, and 1.234 mmol Fe^{2+} E/L, respectively, and for anti-AChE activity is 43.23%. Moreover, 60% of F1 was released within 24 h across the semi-permeable membrane dialysis bag. Additionally, the brine shrimp lethality assay (BSLA) showed a mortality 10%. In conclusion, this study shows that F1 has good antioxidant and anti-acetylcholinesterase activities which can play a promising role as potential antioxidant and anti-AChE therapeutic agent.

ABSTRAK

Rawatan penyakit Alzheimer (AD) telah mendapat banyak perhatian disebabkan oleh keadaan terkini dan jangkaan kelaziman penyakit ini. Ekstrak hidroalkoholik *Centella asiatica* (CAHE), minyak pati serai wangi (LGEO) dan minyak kelapa dara (VCO) yang menunjukkan aktiviti antioksidan dan anti-asetilkolinesterase (anti-AchE) yang diperolehi daripada bahan semulajadi untuk membantu meningkatkan memori pesakit AD. Dalam kajian ini, gambarajah fasa ternari dibina berdasarkan mikromulsi (ME) yang mengandungi CAHE dengan (LGEO atau VCO dan surfaktan bukan ionik, Tween 20 (T20) atau Tween 80 (T80) dengan nisbah yang berlainan. ME yang diformulasikan telah dicirikan untuk mengenal pasti kumpulan berfungsi oleh fourier transformasi inframerah (FTIR), tingkah laku termal oleh kolorimetri pembezaan imbasan (DSC), dan morfologi dan saiz zarah oleh mikroskop daya atom (AFM). Formulasi ME juga diuji ujian aktiviti antioksidan oleh DPPH, ABTS, dan FRAP assay, aktiviti anti-asetilkolinesterase, pembebasan ubat in-vitro, dan ujian sitotoksisiti. Empat gambarajah fasa ternari telah berjaya dibina dan berdasarkan setiap gambarajah ternari, empat formulasi ME yang lutsinar telah dipilih. Keputusan kajian ini menunjukkan bahawa zarah-zarah ME adalah berbentuk sfera dengan pelbagai saiz zarah purata antara 0.1 - 0.2 μm. Ujian kestabilan termodinamik dan jangka panjang yang dijalankan pada suhu bilik menunjukkan bahawa semua formulasi ME mempunyai kestabilan yang baik tanpa sebarang pemisahan. Semua formulasi ME menunjukkan aktiviti antioksida dan anti-AChE yang baik. F1 (Tween 20 / LGEO: CAHE) menunjukkan aktiviti antioksida tertinggi iaitu 79.3%, 93%, dan 1.234 mmol Fe^2 + E / L untuk DPPH, ABTS dan FRAP, dan 43.23% untuk aktiviti anti-ACHE. Tambahan pula, 60% F1 terbebas dalam masa 24 jam di seluruh kantung dialisis membran separuh telap. Di samping itu, “brine shrimp lethality assay” (BSLA) menunjukkan peratusan kematian sebanyak 10%. Kesimpulannya, kajian ini menunjukkan bahawa F1 mempunyai aktiviti antioksida dan anti-asetilkolinesterase yang berpotensi baik dan boleh memainkan peranan yang menjanjikan sebagai ejen terapeutik antioksida dan anti-ACHE.

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LIST OF SYMBOL AND ABBREVIATIONS

%	- percentage
^{o}C	- degree celcius
α	- alpha
β	- beta
ɤ	- gamma
µg	- microgram (s)
µL	- microlitre (s)
µm	- micrometre (s)
μScm^{-1}	- microsiemen per centimetre
cm	- centimetre (s)
cm^{-1}	- per centimetre
g	- gram (s)
h	- hour (s)
Hz	- Hertz
I%	- Scavenging effect
kDa	- kilodalton
kg	- kilogram (s)
min	- minute (s)
mg	- milligram (s)
mL	- millilitre (s)
µM	- micromolar
mM	- milimolar
M	- molarity
mPa*s	- millipascal-second
nm	- nanometre (s)
rpm	- revolutions per minutes
sec	- second (s)
ABTS	- 2,2′-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid)

AD	- Alzheimer's disease
ACh	- Acetylcholine
AChE	- Acetylcholinesterase
AFM	- Atomic force microscopy
ATCI	- Acetylthiocholine
BBB	- Blood brain barrier
BChE	- butyrylcholinesterase
CA	- *Centella asiatica*
CAHE	- *Centella asiatica* hydroalcoholic extract
ChAT	- Cholineacetyltranferase
CNS	- Central nervous system
CoA	- Coenzyme A
CSF	- Cerebrospinal fluid
DDS	- Drug delivery system
DPPH	- 2,2-diphenyl-1-picrylhydrazyl
DSC	- Differential scanning calorimetry
DTNB	- 5,5′-Dithiobis [2-nitrobenzoic acid]
FRAP	- Ferric reducing assay power
FTIR	- Fourier transfrom infrared
HCl	- Hydrocloric acid
IR	- Infrared
LGEO	- Lemongrass essential oil
ME	- microemulsions
ME-CAHE	- Microemulsion containing *Centella asiatica* hydroalcoholic extract
MRI	- Magnetic resonance imaging
NMJ	- Neuromuscular junction
O/W	- Oil-in-water
PBS	- Phosphate buffered saline
ROS	- reactive oxygen species
TJ	- Tight junction
TPTZ	- 2,4,6-Tris(2-pyridyl)-s-triazine
UV	- Ultra-violet
VCO	- Virgin coconut oil
W/O	- Water in oil

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Conferences

1. **Muhammad Ihsan Ghazali,** Faridah Kormin, Muhammad Sohail Latif, Sameera Abbas, Nor Shafawati Mohd Shafie, Mohd Fadzelly Abu Bakar, Ida Idayu Muhamad. Phase behavior study of lemongrass essential oil/virgin coconut oil containing *Centella asiatica* hydroalcoholic extract with tween 20/tween 80. International Research and Innovation Symposium Exposition 2018 (RISE 2018) from 12 - 13 November 2018 at Dewan Sultan Ibrahim, Universiti Tun Hussein Onn Malaysia (UTHM).

CHAPTER 1

INTRODUCTION

1.1 Background of study

Alzheimer's disease (AD) remains as the most common primary degenerative dementia disorder for elderly people (Tarawneh & Holtzman, 2012; Korolev, 2014). AD is a clinical syndrome characterized by diverse behavioral, cognitive and emotional impairments in addition to the progressive deterioration of short-term memory and other higher cortical function (Chapman *et al*., 2006). According to the World Health Organization (WHO), the prevalence of AD was estimated to be about 24.3 million people and every year 4.6 million new cases are reported. Also, 60-80% of cases of the AD are reported worldwide per year. The number of people affected is expected to be doubled every 20 years (Cornutiu, 2015).

The neurodegeneration occurring in AD affects multiple neurotransmitters, the cholinergic system has received the higher attention (Mega, 2000). The AD early onset and progression are related to the increased level 0of acetylcholinesterase (AChE) enzyme which catalyzes the rapid hydrolysis of neurotransmitter, acetylcholine (ACh), and thus leading to the cholinergic deficit (Čolović *et al.*, 2013). The imbalance between the amounts of free radicals produced and their elimination by the body's defense mechanism ultimately also contributes in the accumulation of free radicals or reactive oxygen species (ROS) in the brain tissue.

Bioactive phytoconstituents resulted from isolation of flora and fauna always been an important reservoir of drugs. The majority of compounds affecting cholinergic neurotransmission and aiding protection against AD are primarily derived from plants sources such as phenol, terpenoid and flavanoid (Bhattarcharjee, 2018). Indeed, several pharmacological screenings showed the efficacy of *Centella asiatica* (CA) extract alone or in combination along their active constituent to encounter AD

(Bhattarcharjee, 2018). This is because CA contains certain compounds with significant antioxidant and anti-AChE properties (Nour *et al*., 2014). Hence, more scientists have become inquisitive of the chemical moieties of this species (Suganthy & Pandima Devi, 2016). The CA extract has been recommended by pharmacopeia regarding the function as an Ayurvedic medicine (Rao *et al*., 2007). In fact, several research reports in the scientific literature have shown the neuroprotective effects of CA (Rao *et al*., 2005; Rahman *et al*., 2012; Nour *et al*., 2014).

Previous results showed approximately 40% of the extracts have solubility problem that makes the compounds difficult to cross the cell membrane (Pardridge, 2001; Upadhyay, 2014; Parth, *et al*., 2016). Surprisingly , the issue of the administration of poorly water-soluble extracts can solved by using as a delivery carrier to carry the extract (Van Hoogevest, 2011). The extract carriers not only encapsulate the extract and protect it from biological defense mechanism, but also help to release the extracts in a controlled manner at the target site. There are several types of carriers currently being investigated, including microemulsion (ME), nanoemulsion, micelles, liposomes, polymersomes, dendrimers, polymeric nanoparticles, solid lipid nanoparticles and nanostructured lipid carriers (Kaul, 2018). Most of these carriers are self-assembled structures from ubiquitous process in the cellular system. The functionalization of ME showed the advantages that is also increasingly integrated into drug delivery system.

Microemulsion is thermodynamically stable, transparent or translucent, and isotropic dispersion with particles sizes ranging from 10 nm to 100 nm. It consists of oil, surfactant, and water, typically in conjunction with a cosurfactant (Dubey *et al*., 2017). It also plays an essential role in the delivery of herbal extract, drug, and pharmaceutical agents besides the delivery of the extract into specific tissues and cells (Parth, *et al*., 2016). There are several advantages of ME over other traditional drug carriers including the possibility of controlled drug release and drug targeting, increased drug stability, good availability, low cytotoxicity, good production scalability, avoidance of organic solvents in the preparation process and potential application spectrum (Pardridge, 2001; Tiwari *et al.*, 2012).

1.2 Problem statement

The decreasing cognitive function in elderly people with AD has become a common problem that has affected human life and health in society (Anekonda & Reddy, 2005). The AD is a neurodegenerative disease that is associated with hippocampal atrophy and loss of function of cortical neurons in the brains (Lillrank, 2007). AChE is a key factor that leads to decreased level of ACh in the brain which is considered as one of the pathogenesis of AD. The AChE breaks down the neurotransmitter ACh into choline and acetate. According to Golan *et al*. (2011), AChE has a high enzymatic activity that is capable to hydrolyze 4×10^5 of ACh molecules in one minute.

Antioxidant and anti-AChE agents has been produced, applied for AD and has been commercialized in pharmaceutical industry. The commercial anti-AChE agents such as galanthamine, donepezil, rivastigmine and tracrine have the ability to stop progression of the disease, however they are causing few side effects (Yiannopoulou & Papageorgiou, 2013). The side effects of the common anti-AChE including dizziness, nausea and stomach ache (Čolović *et al*.,2013). The development of new potential inhibitor that control the activity of acetylcholinesterase with minimum side effects is need. However, transportation of molecules through blood brain barrier (BBB) is still a major challenge because of their size are more than 300 nm (Upadhyay, 2014). The presence of BBB letting not more than 2% of small size drug molecules and blocking all the large molecules, is the most important physical obstacle on the way to accomplish the task of successful drug delivery to AD brains. Till now, most of the drugs have low bioavailability due to their low solubility in water and high molecular size. Solubilization of poor soluble drugs is a major challenge in screening studies of new potential inhibitor as well as in formulation design and development (Bittner & Mountfield, 2002). Thus, it has attracted more scientists to discover new effective antioxidant and anti-AChE agents to treat the AD.

Until now, there are no reports documented on using virgin coconut oil (VCO) and lemongrass essential oil (LGEO) as a carrier for *Centella asitica* hydroalcoholic extract (CAHE) as antioxidant and anti-AChE. This research explored an efficient drug delivery system based on ME having the ability to act as the potential antioxidant and anti-AChE agent. New ME formulations may be used to be a potential anti-AChE agent.

1.3 Objectives of study

The objectives of this study are:

1. To construct the phase behavior of the ME system.
2. To characterize the ME formulation in various surfactant and oil.
3. To evaluate antioxidant and anti-acetylcholinesterase activity, drug release and cytotoxicity of various ME formulation.

1.4 Scope of study

Through this research, scopes are taken into consideration to achieve the objectives. The scopes of the study are mentioned as below:

1.4.1 Extraction and construction of ternary phase diagram ME formulation.

The ternary phase diagram was investigated by their phase behaviour. The MEs were prepared in different ratios of VCO and LGEO as an oil phase with tween 20 and tween 80 act as a surfactant and water. By observing the phase behaviour of the mixture, the ternary phase diagram was constructed and the appropriate and stable ME was selected.

1.4.2 Characterization of ME formulations.

The prepared ME formulations were characterized for the identification of functional groups by FTIR, thermal behaviour by DSC, morphology and particle size by AFM. The thermodynamic stability study was performed based on heating-cooling, freeze-thaw cycle, and centrifugation. The long-term stability study was carried out based primarily on physical observation followed by measurement of pH, conductivity, turbidity, and viscosity.

1.4.3 Evaluation of antioxidant activity, anti-acetylcholinesterase activity, drug release and cytotoxicity of various ME formulations

The antioxidant and anti-AChE potential of ME formulations were determined by antioxidant assays (DPPH, ABTS, and FRAP) and AChE inhibition activity assays, respectively. Finally, the effectiveness of the ME formulation as drug delivery system was performed using *in-vitro* dialysis bag technique. The cytotoxicity of various ME formulations was determined by brine shrimps lethality assay test (BSLA).

1.5 Significance of the study

The controversy about the safety of common Alzheimer's disease drug will never come to an end as scientists have begun to recognize the side effects of anti-AChE drugs and published in the literature. Plants have gained popularity as a source of herbal medicine for treatment of AD. *Centella asiatica* hydroalcoholic extract (CAHE) has shown to improve cognitive function, besides acting as anti-AChE, antioxidant agent (Hashim *et al*., 2011; Orhan *et al*., 2012). Several active compounds from CAHE of this plant including triterpene, phenolic and flavonoid were found to be an antioxidant and anti-AChE (Soumyanath, *et* al., 2012). It is believed that the microemulsion (ME) can increase the effectiveness of the therapeutic agent as a drug carrier. Although, ME are promising tools for delivery of therapeutic devices to the brain via several routes of administration (Fonseca-Santos *et al*., 2006). The encapsulation also protects the drugs or extract from interaction with microorganism/bacteria and improve stability. In addition, ME are convenient and easy to formulate. As more efficient drug delivery systems and developed formulations become available, the health professionals will have a better choice that will target specific cells. In this study, ME formulation containing CA hydroalcoholic extract was prepared and characterized. The ME containing *Centella asiatica* hydroalcoholic extract was prepared using VCO and LGEO as the oil phase, Tween 20 and Tween 80 were used as the surfactant. It is expected that the results of this study would be helpful to further assess the role of the ME containing *Centella asiatica* hydroalcoholic extract as potential nanomedicine for AD treatment.

CHAPTER 2

LITERATURE REVIEW

2.1 Overview of Alzheimer's Disease (AD)

Alzheimer's disease is type of a neurodegenerative disorder which is related to progressive cognitive and functional deficits, mostly experienced by people aged 65 years and older (Apostolova, 2016). The AD is not part of the normal aging process rather it is caused by progressive loss of nerve cells in the brain. Nerve cells are important for normal brain functions such as thinking, memory, spatial orientation, verbal communication and decision making (Fonseca-Santos *et al*., 2006). The patients of AD suffer from impaired brain function that interferes with their normal daily activities. The AD can also be characterized by impaired neuronal signaling, leading to a slow and progressive decline in cognitive function and behaviour (Ismail *et al*., 2013). The AD is also known as The Long Goodbye, the disease typically spent over 10 to 20 years. Despite intensive research carried out in past decade, the AD is still un-understandable and incurable, thus early detection and treatment is the only option to manage the disease effectively (Koh Abdullah, 2008, Fonseca-Santos *et al*., 2006).

Pathologically, Alzheimer's brain can be characterized by imaging studies like MRI or CT scans. The brain of a person with AD appears to be getting smaller and shrinking. The most prominent regions in the brain affected by AD include cerebral cortex, hippocampus, entorhinal cortex and ventral striatum as shown in Figure 2.1 (a) (Lilrank, 2007). Besides, the cerebrospinal fluid (CSF) fills in the cavities of the brain and the cavities become enlarged as shown in Figure 2.1(b). In the parenchyma of Alzheimer's brain, there are extracellular aggregates of Amyloid-β (Aβ) peptide in senile plaques (Aishwarya & Sumathi, 2016), intracellular accumulation of tau

proteins (Mourtas *et al*., 2014), and progressive synaptic dysfunction (Bernardi *et al*., 2012; Herran *et al*., 2013).

The AD begins slowly and can be identified as early onset and late onset based on the appearance of symptoms. In early onset, forgetfulness is considered as the first sign of the AD. People in the early stages of AD might have trouble in remembering recent events, activities, or the names of familiar people or things. Simple math problem can be difficult to solve. However, as the disease progresses, the additional symptoms like behavioural changes (Kakkar & Kaur, 2011), forgetting the well-known places and family members and friends, being unable to track or follow the directions, constantly asking the same questions and lack of care due to personal safety and hygiene, also appear.

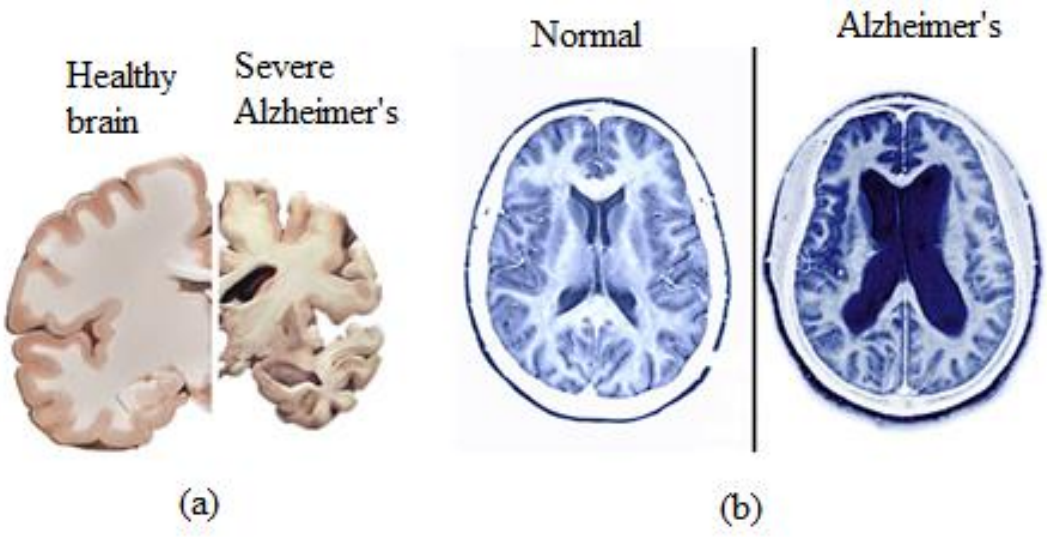

Figure 2.1: Images of (a) The cross-section of normal human brain and Alzheimer brain (b) MRI images of Alzheimer brain tissue shrinks which contains cerebrospinal fluid (Jagadeesh Kumar, 2017).

REFERENCES

Abd Gani, S. S., & Adisah, S. Z. (2014). Phase behaviour study of swiftlet nest using virgin coconut oil with non-ionic surfactants. *Malaysian Journal of Analytical Sciences*, 19(1), 184 - 193.

Abdulkarim, M. F., *et al*., (2011). Study of pseudoternary phase diagram behaviour and the effect of several tweens and spans on palm oil esters characteristics. *International Journal of Drug Delivery,* 3, 95 - 100.

Adedapo, A. A., Jimoh, F. O., Afolayan, A. J., & Masika, P. J. (2009). Antioxidant properties of the methanol extracts of the leaves and stems of *Celtis africana*. *Records of Natural Products*, 3(1), 23-31.

Adedayo, B. C., Oboh, G., Oyeleye, S. I., Ejakpovi I. I., Boligon, A. A., & Athaye, M. L. (2015). Blanching alters the phenolic constituents and in-vitro antioxidant and anticholinesterase properties of fireweed (*Crassocephalum crepidioides)*. *Journal Taibah University Medical Science*, 10(4), 419 - 426.

Ames, B. N., Shigenaga, M. K., & Hagen, T. M. (1993). Oxidants, antioxidants, and the degenerative diseases of aging. *Proceedings of the National Academy of Sciences of the United States of America*, 90(17), 7915-7922.

Amidon, G. L., Lennernäs, H., Shah, V. P., & Crison, J. P. (1995). A theoretical basis for a biopharmaceutic drug classification: the correlation of in vitro drug product dissolution and in vivo bioavailability. *Pharmaceutical Research,* 12(3), 413 - 420.

Anekonda, T. S., & Reddy, H. (2005). Can herbs Provide a New Generation of Drugs for Treating Alzheimer's Disease. *Brain Research Review,* 50, 361- 376.

Apostolova, L. G. (2016). Alzheimer Disease. *Continuum : Lifelong Learning in Neurology*, 22(2), 419 - 434.

Aishwarya, V., & Sumathi, T. (2016). Mitigating effect of chrysin loaded solid lipid nanoparticles against Amyloid β25-35 induced oxidative stress in rat hippocampal region: An efficient formulation approach for Alzheimer's disease. *Neuropeptides*, 58, 111 - 125.

Arlee, R., Suanphairoch, S., & Pakdeechanuan, P. (2013). Differences in chemical components and antioxidant-related substances in virgin coconut oil from coconut hybrids and their parents. *International Food Research Journal*, 20(5), 2103 - 2109.

Awad, R., Levac D., Cybulska, P., Merali, Z., Trudeau, V.L., & Arnason, J.T. (2007). Effects of traditionally used anxiolytic botanicals on enzymes of the γ-aminobutyric acid (GABA) system. *Canadian Journal of Physiology and Pharmacology*, 85(9), 933 - 942.

Awang, D. V. C. (2009). *Tyler Herbs of Choice: The Therapeutic Use of Phytomedicinals*. 3rd ed. Boca Raton: CRC Press.

Aziz, Z. A., Davey, M. R., Power, J. B., Anthony, P., Smith, R. M., & Lowe, K. C. (2007). Production of asiaticoside and madecassoside in Centella asiatica in vitro and in vivo. *Biologia Plantarum*, *51*(1), 34–42.

Baker, B. P., & Grant J. A. (2018). Lemongrass oil profile. *New York IPM Program*, 1 - 12.

Baki, G., Chaudhari, S., Gaudana, R., & Boddu, S. H. S. (2015). Selection of excipients based on the biopaharmaceutics classification system of drugs. *Excipient Applications in Formulation Design and Drug Delivery*, 351 - 383.

Baravalia, Y., Vaghasiya, Y., & Chanda, S. (2012). Brine shrimp cytotoxicity, anti-inflammatory and analgesic properties of woodfordia fruticosa Kurz flowers. *Iranian Journal Of Pharmaceutical Research*, 11(3), 851- 61.

Barbu, E., Molnar, E., Tsibouklis, J., & Gorekcki, D. C. (2009). The potential for nanoparticle-based drug delivery to the brain: overcoming the blood-brain barrier. *Expert Opinion Drug Delivery 6*, 553 - 565.

Baynes, J. W., & Thorpe, S. R. (1999). Role of oxidative stress in diabetic complications: a new perspective on an old paradigm. *Diabetes*, 48, 1 - 9.

Benzie, I. F. F. & Strain, J. J (1999). Ferric reducing/antioxidant power assay: Direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. *Method in Enzymology*, 299, 15 - 27.

Bernardi, A., *et al*., (2012). Indomethacin-loaded lipid-core nanocapcules reduce the damage triggered by Aβ1-42 in Alzheimer's disease models. *International Journal of Nanomedicine*, 7, 4927 - 4942.

Bhattarcharjee, A. (2018). Anticholinesterase potential of phytoextracts and their bioactive compound: a promising therapeutic agent against Alzheimer's disorder. *MOJ Tumor Research*, 1(2), 83 - 87.

Bickel, U., Yoshikawa, T., & Rardridge, W. M. (2001). Delivery of peptides and proteins through the blood-brain barrier. *Advanced Drug Delivery Reviews*, 46, 247 - 279.

Bittner B., & Mountfield, R. J. (2002). Intravenous administration of poorly soluble new drug entities in early drug discovery: the potential impact of formulation on pharmacokinetic parameters. *Current Opinion in Drug Discovery and Development*, 5, 59 -71.

Briuglia, M. L., Rotella, C., McFarlane, A., Lamprou, D. A. (2015). Influence of cholesterol on liposome stability and on in vitro drug release. *Drug Delivery and Translational Research*, 5(3), 231 - 242.

Bumajdad, A., & Eastoe, J. (2004). Conductivity of water-in-oil microemulsions stabilized by mixed surfactants. *Journal of Colloid and Interface Science*, 274(1), 268 – 276.

Chaiyana, W., Saeio, K., Hennink, W. E., & Okonogi, S. (2010). Characterization of potent anticholinesterase plant oil based microemulsion. *International Journal of Pharmaceuticals*, 401, 32 - 40.

Chakraborty, K, & Paulraj, R. (2010). Sesquiterpenoids with free radicals cavenging properties from marine macroalga *Ulva fasciata* Delile. *Food Chem*istry, 122, 31 - 41

Chapman, D. P., Marshall Williams, S., Strine, T. W., Anda, R. F., & Moore, M. J. (2006). Dementia and its implications for public health. *Preventing Chronic Disease*, *3*(2), 1 - 13.

Chen, H., Chang, X., Weng, T., Zhao, X., Gao, Z., Yang, Y., Xu, H., & Yang, X. (2004). A study of microemulsion systems for transdermal delivery of triptolide. *Journal of Controlled Release*, 98(3), 427 - 436.

Cheng, S., Song, W., Yuan, X., & Xu, Y. (2017). Acetylcholinesterase revealed by microsecond molecular dynamics simulations. *Scientific Report*, 7 (3219), 1 - 12.

Chew, K. K., Ng, S. Y., Thoo, Y. Y., Khoo, M. Z., Wan Aida, W. M., Ho, C. W. (2011). Effect of ethanol concentration, extraction time and extraction temperature on the recovery of phenolic compounds and antioxidant capacity of *Centella asiatica* extracts. *International Food Reseach Journal*, 18, 571 - 578.

Chiavaro, E. (2015). *Differential scanning colorimetry: Applications in fat and oil technology*. Boca Raton: CRC Press.

Cho, Y. (2008). Formulation of co-surfactant-free O/W microemulsion using Nonionic Surfactant Mixtures. *Journal of Food Sciences*, 73(3), 115 - 121.

Chong, N. J., & Aziz, Z. (2011). A systematic review on the chemical constituents of *Centella asiatica*. *Research Journal of Pharmaceutical, Biological and Chemical Science*, 2(3), 445 - 459.

Čolović, M. B., Krstić, D. Z., Lazarević-Pašti, T. D., Bondžić, A. M., & Vasić, V. M. (2013). Acetylcholinesterase inhibitors: pharmacology and toxicology. *Current Neuropharmacology*, *11*(3), 315 - 335.

Cornutiu, G. (2015). The epidemiological scale of Alzheimer's disease. *Journal of Clinical Medicine Research*, *7*(9), 657 - 666.

Deepak, S. N., & Vedha Hari, B. N. (2013). Optimization, development and evaluation of microemulsion for the release of combination of guaifenesin and phenylephrine. *Journal of Applied Pharmaceutical Science*, 3(9), 48 - 56.

Dhar M. L., Dhar M. C., Dhawan B. N., Mehrotra B. N., & Ray C. (1968). Screening of Indian plants for biological activity: Part I. *Indian Journal of Experimental Biology*, 2, 232 - 247.

Doknark, S., Mingmalairak, S., Vattanajun, A., Tantisira, B., & Tantisira, M.H. (2014). Study of ameliorating effects of ethanolic extract of *Centella asiatica* on learning and memory deficit in animal models. *Journal of the Medicinal Association of Thailand*, 97, 68 - 76.

Droge, W. (2002). Free radicals in the physiological control of cell function. *Physiological Reviews*, 82, 47 - 95.

Duan, X., Li, M., Ma, H., Xu, X., Jin, Z., & Liu, X. (2016). Physicochemical properties and antioxidant potential of phosvitin-resveratrol complexes in emulsion system. *Food Chemistry*, 206, 102 - 109.

Dubey, S., Sharma, R., Mody, N., & Vyas, S. P. (2017). Novel carriers and approaches: insight for psoriasis management. *Nanostructures for Novel Therapy: Synthesis, Characterization and Applications*, 2017, 657 - 684.

Dvir, H., Silman, I., Harel, M., Rosenberry, T. L., & Sussman, J. L. (2010). Acetylcholinesterase: From 3D structure to function. *Chemico-Biological Interactions, 187*(1-3), 10 - 22.

Eastoe, J. (2005). Microemulsion. *Colloid Science: Principles, Methods and Applications*, 77 - 97.

Ellman, G. L., Courtney, K. D., Andres, V. J., & Feather-Stone, R. M. (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochemical Pharmacology*, 7, 88 - 95.

Evans, P. H. (1993). Free radicals in brain metabolism and pathology. *British Medical Bulletin*, 49(3), 577-587.

Fang, L., Chen, M., Liu, Z., Fang, X., Gou, S., & Chen, L. (2016). Ferulic acid-carbazole hybrid compounds: combination of cholinesterase inhibition, antioxidant and neuroprotectivion as multifunctional anti-Alzheimer agents. *Bioorganic & Medicinal Chemistry*, 24, 886 - 893.

Feng, J. L., Wang, Z. W., Zhang, J., Wang, Z. N., & Liu, F. (2009). Study on food-grade vitamin E microemulsion based on nonionic emulsifiers. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 339, 1 - 6.

Fernando W. M., Martins I. J., Goozee K. G., Brennan C. S., Jayasena V., & Martins R. N. (2015). The role of dietary coconut for the prevention and treatment of Alzheimer's disease: potential mechanisms of action. *The British Journal of Nutrition*, 114(1), 1 - 14.

Fitriani, L., Algariat, F., Fortunella, F., & Lucida, H. (2017). Formulation and evaluation of microemulsion from chloroform extract of tomato (*Solanum Lycopersicum* L.) *Der Pharmacia Lettre*, 9(2), 48 - 57.

Fonseca-Santos, B., Gremiao, M. P. D., & Chorilli, M. (2015). Nanotechnology-based drug delivery systems for treatment of Alzheimer's disease. *International Journal of Nanomedicine*, 10, 4981 - 5003.

Floyd, R. A. (1999). Antioxidants, oxidative stress, and degenerative neurological disorders. *Proceedings of the Society for Experimental Biology and Medicine*, 222(3), 235 - 245.

Frahm, S., Antolin-Fontes, B., Görlich, A., Zander, J. F., Ahnert-Hilger, G., & Ibañez-Tallon, I. (2015). An essential role of acetylcholine-glutamate synergy at habenular synapses in nicotine dependence. *eLife*, 4, 1 - 31.

Fukuto, T. R. (1990). Mechanism of action of organophosphorus and carbamate insecticides. *Enviromental Health Perspectives*, 87, 245 - 254.

Garti, N., Aserin, A., Tiunova, I., & Fanum, M. (2000). A DSC study of water behavior in w/o microemulsions stabilized by sucrose esters and butanol. *Colloid Surface*,120,1 - 18.

Garti, N., Spernath, A., Aserin, A., & Lutz, R. (2002). Nano-sized self-assemblies of nonionic surfactants as solubilization reservoirs and microreactors for food systems. *Soft Matter*, 1, 206 - 218.

Ghosh, K., Indra, N., & Jagadeesan, G. (2017). The ameliorating effect of *Centella asiatica* ethanolic extract on albino rats treated with isoniazid. *Journal of Basic And Clinical Physiology And Pharmacology*, 28(1), 67 - 77.

Ghribia, L., Ghouilaa, H., Omrib, A., Besbesb, M., & Janneta, H. B. (2014). Antioxidant and anti-acetylcholinesterase activities of extracts and secondary metabolites from Acacia cyanophylla. *Asian Pacific Journal Of Tropical Biomedicine*, 4(1), 417 - 423.

Gulcin, I., Koksal, K., Elmastas, M., & Aboul-Enein, H. Y. (2007). Determination of *in vitro* antioxidant and radical scavenging activity of *Verbascum oreophilum* C. Koch Var Joannis (Fam. Scrophulariaceae). *Research Journal of Biological Sciences* 2(3), 372-382.

Gupta, S., & Prakash, J. (2008). Influence of antioxidant components on antioxidant activity of dehydrated green leafy vegetables. *Food Science Technology Research*, 14(1), 104 - 109.

Gupta, S., & Prakash, J. (2009). Studies on Indian green leafy vegetables for Their antioxidant activity. *Plant Foods for Human Nutrition*, 64(1), 39 - 45.

Gunther, B., & Wagner, H. (1996). Quantitative determination of triterpenes in extracts and phytopreparations of *Centella asiatica*(L.) Urban. *Phytomedicine*, 3(1), 59 - 65.

Gutierrez-Mecinas, M., Davis, O., Polgár, E., Shahzad, M., Navarro-Batista, K., Furuta, T., … Todd, A. J. (2019). Expression of Calretinin Among Different Neurochemical Classes of Interneuron in the Superficial Dorsal Horn of the Mouse Spinal Cord. *Neuroscience*, 398, 171–181.

Halliwell, B. (1989). Oxidants and the central nervous system: some fundamental questions. Is oxidant damage relevant to Parkinson's disease, Alzheimer's disease and traumatic injury or stroke?. *Acta Neurologica Scandinavica Supplementum*, 126, 23 - 33.

Halliwell, B., & Gutteridge, J. M. C. (2007). *Free radicals in Biology and Medicine*, 4th ed. Oxford University Press, New York.

Handayani, A., Yolanda, S., & Kodariah, R. (2018). *Centella asiatica* ethanol extract increases hippocampal brain derived neurotrophic factor in male wistar rats. *Universa Medicina*, 37(2), 143 - 149.

Hashim, P., Sidek, H., Helan, M. M., Sabery, A., Palanisamy, U. D., & Ilham, M. (2011). Triterpene composition and bioactivities of *Centella asiatica*. *Molecules*, 16, 1310 - 1322.

Herran, E., Perez-Gonzalez, R., Igartua, M., Pedraz, J. L., Carro, E., & Hernandez, R. M. (2013). VEGF-releasing biodegradable nanospheres administered by craniotomy: A novel therapeutic approach in the APP/Ps1 mouse model of Alzheimer's disease. *Journal of Controlled Release*, 170, 111 - 119.

Ho, Y. S., So, K. F., & Chang, R. C. C. (2010). Anti-aging herbal medicine-How and why can they be used in aging-associated neurodegenerative diseases?. *Ageing Research Review*, 9 (3), 354 - 362.

Huang, W. J., Zhang, X., & Chen, W. W. (2016). Role of oxidative stress in Alzheimer's disease (Review). *Biomedical Reports*, 4, 519 - 522.

Ibrahim, U. K., Muhammad, I. I., & Salleh, R. M. (2011). The effect of pH on color behavior of Brassica oleracea anthocyanin. *Journal of Applied Sciences*, 11, 2406 - 2410

Idham, Z., Muhamad, I. I., Mohd Setapar, S. H., & Sarmidi, M. R. (2012). Effect of thermal processes on roselle anthocyanins encapsulated in different polymer matrices. *Journal of Food Processing and Preservation,* 36 (2), 176 - 184.

Ismail, M. F., Elmeshad, A. N., & Salem, N. A. (2013). Potential therapeutic effect of nanobased formulation of rivastigmaine on rat model of Alzheimer's disease. *International Journal of Nanomedicine*, 8, 393 - 406.

Jamil, S. S., Nizami, Q., & Salam, Mehboobus. (2007). *Centella asiatica* (Linn.) urban *óA Review. Natural Product Radiance,* 6(2), 158 - 170.

Jagadeesh Kumar, P. S. (2017). Computer-aided therapeutic of Alzheimer's disease eulogizing pattern classification and deep learning protruded on tree-based learning method. *Progress in Advanced Computing and Intelligent Engineering*, 103 - 113.

Jefferys, D. B. (2001). The regulation of medical devices and the role of Medical Devices Agency. *British Journal of Clinical Pharmacology*, 52(3), 229 - 235.

Jeirani. Z., Mohamed Jan, B., Si Ali, B., Noor, I. M., See, C. H. & Saphauchart, W. (2013). Formulation and phase behaviour study of nonionic triglyceride microemulsion to increase hydrocarbon production. *Industrial Crops and Products*, 43, 15 - 24.

Jena, S. K., Singh, C., Dora, C. P., & Suresh, S. (2014). Development of tamoxifend-phospholipid complexed: novel approach for improving solubility and bioavailability. *International Journal of Pharmaceutical*, 473(1-2), 1 - 9.

Johnson, A. W. (1999). *Invitation to Organic Chemistry*. Massachusetts: John and Bartlett Publisher.

Kaul, S., Gulati, N., Verma, D., Mukherjee, S., & Nagaich, U. (2018). Role of nanotechnology in cosmecerticals: A review of recent advances. *Journal of Pharmaceutics*, 2018, 1 - 19.

Kakkar, V., & Kaur I. P. (2011). Evaluating potential of curcumin loaded solid lipid nanoparticles in aluminium induced behavioural, biochemical and histopathological alterations in mice brain. *Food and Chemical Toxicology*, 49, 2906 - 2291.

Kesornbuakao, K., Yongsirasawad, K., Rattanakom, S., & Yusarin, P. (2017). Antioxidant activity of bua-bok(*Centella asiatica*) ethanolic extract loaded gelatin nanoparticles. *Journal of Science and Technology Ubon Ratchathani Universtiy: Special Issue Septemper 2017*, 198 - 202.

Khadka, P., Ro, J., Kim, H., Kim, I., Kim, J. T., Kim, H., Cho, J. M., Yun, G., & Lee, J. (2014). Pharmaceutical particle technologies: an approach to improve drug solubility, dissolution and bioavailability, *Asian Journal of Pharmaceutical Sciences* ,9, 304 - 316.

Kido Y., Tamai, I., Okamoto, M., Suzuki, F., & Tsuji, A. (2000). Functional clarification of MCT1-mediated transport of monocarboxylic acids at the blood-brain barrier using *in vitro* cultured cells and *in vivo* BUI studies. *Pharmaceutical Research*, 17(1), 55 - 62.

Koh Abdullah, R. A. (2008). *Alzheimer penyebab nyanyuk*. Retrieved on September 21, 2008, http://ww1.utusan.com.my/utusan/info.asp?y=2008&dt=0921&sec=Kesihatan&pg=kn_01.htm.

Korolev, I. O. (2014). Alzheimer's disease: A clinical and basic science review. *Medical Student Research Journal*, 4, 24 - 34.

Kovarik, Z., Radic, Z., Berman, H. A., Simeon-Rudolf, V., Reiner, E. & Taylor, P (2003). Acetylcholinesterase active centre and gorge conformations analysed by combinatorial mutations and enantiomeric phosphonates. *Biochemical Journal*, 373, 30 - 40.

Kuroda, M., Mimaki, Y., Harada, H., Sakagami, H., & Sashida, Y. (2001). Five new triterpene glycosides from *Centella asiatica*. *Natural Medicines*, 55(3), 134 - 138.

Lawal, O. A., Ogundajo, A. L., Avoseh, N. O., & Ogunwande, I. A. (2017). Cymbopogon Citratus. *Medicinal Spices and Vegetables from Africa*, 2017, 397 - 423.

Lawrence, M. J., & Rees, G. D. (2000). Microemulsion-based media as novel drug delivery systems. *Advanced Drug Delivery Reviews*, 48, 89 - 121.

Lee, G. S., Lee, D. H., & Pyo, H. B. (2011). Preparation and characterization of encapsulation of multiple lipid carrier (MLC) using vegetable fat. *Journal of Industrial and Engineering Chemistry*, 17, 421 - 426.

Li, H., & Harlow, M. L. (2014). Individual synaptic vesicles from the electroplaque of Torpedo californica, a classic cholinergic synapse, also contain transporters for glutamate and ATP. *Physiological reports*, *2*(1), 1 - 9.

Lillrank, S. M., Collins, C., & Levitt, P. (2007) *Psychological Disorder: Alzheimer's Disease and Other Dementia.* New York: Chelsea House Publisher.

Lin, C. C., Wu, S. J., Wang, J. S., Yang, J. J., & Chang, C. H. (2001). Evaluation of the antioxidant activity of legumes. *Pharmaceutical biology*, 39(4), 300 - 304.

Lin, P. C., Lin, S., Wang, P. C., & Sridhar, R. (2014). Techniques for physicochemical characterization of nanomaterials. *Biotechnology Advances*, 32(4), 711 - 726.

Lipinski, C. A., Lombardo, F., Dominy, B. W., & Feeney, P. J. (2001). Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Advanced Drug Delivery Reviews*, 46(1 - 3), 3 - 26.

Liu, W., Sun, D., Li, C., Liu, Q., & Xu, J. (2006). Formation and stability of paraffin oil-in-water nano-emulsions prepared by the emulsion inversion point method. *Journal of Colloid and Interface Science*, 303(2), 557 - 563.

Lobo, V., Patil, A., Phatak, A., & Chandra, N. (2010). Free radicals, antioxidants and functional foods: Impact on human health. *Pharmacognosy reviews*, *4*(8), 118 - 126.

Loeffler, D. A., Connor, J. R., Juneau, P. L., Snyder, B. S., Kanaley, L., DeMaggio, A. J., Nguyen, H., Brickman, C. M., & Lewitt, P. A. (1995). Transferrin and iron in normal, Alzheimer's disease and Parkinson's disease brain regions. *Journal of Neurochemistry*, 65(2), 710 - 724.

Mandal, S., Mandal S. S., Surti, N., & Patel, V. B. (2009). Design and development of Saquinavir microemulsion for the oral bioavailability enhancement. *International Journal of PharmTech Research*,1, 1442 - 1448.

Manoharan, S., Guillemin, G. J., Abiramasundari, R. S., Mohamed Essa, M., Akbar, M., & Akbar. M. D. (2016). The role of reactive oxygen species in the pathogenesis of Alzheimer's disease, Parkinson's disease, and Huntington's disease: A mini Review. *Oxidative Medicine and Cellular Longevity*, 2016, 1 - 15.

Marinova, K. G., Alargova, R. G., Denkov, N. D., Velev, O. D., Petsev, D. N., Ivanov, I. B., & Borwankar, R. P. (1996). Charging of Oil-Water interfaces due to spontaneous adsorption of Hydroxyl Ions. *Langmuir*, 12, 2045 - 2051.

Martins, P., Sbaite, P., Benites, C., & Maciel, M. (2011). Thermal Characterization of orange, lemongrass and basil essential oils. *Chemical Engineering Transactions*, 24, 463 - 468.

Mathialagan, R., Nour, A. H., Sulaiman, A. Z., Nour, A. H., & Raj, T. (2014). Comparison of chemical composition and toxicity of essential oils from lemongrass (*Cymbopogon citratus*) extracted with microwave-assisted hydrodistillation (MAH) and conventional hydrodistillation (HD) methods. *Journal of Advances in Natural Sciences*, 1(1), 10 - 15.

Maulidiani, Abas, F., Khatib, A., Shaari, K., & Lajis, N. H., (2014). Chemical characterization and antioxidant activity of three medicinal Apiaceae species. *Industrial Crops and Products*, 55, 238 - 247.

McClements, D. J. (2012). Nanoemulsions versus microemulsios: terminology, differences, and similarities. *Soft Matter*, 8, 1719 - 1729.

McCorry, L. K. (2007). Physiology of the Autonomic Nervous System. *American Journal of Pharmaceutical Education*, *71*(4), 78.

McDermott, J.H. (2000). Antioxidant nutrients: current dietary recommendations and research update. *Journal of the American Pharmaceutical Association*, 40(6), 785 - 799.

McLaughlin J, Chang C and Smith D (1991). Proceedings of the 18 th National Seminar and UNESCO Workshop on Natural Products. Institute of Advance Studies, University of Malaysia, Malaysia.

Meena, H., Pandey, H. K., Pandey, P., Arya, M. C., & Ahmed, Z. (2012). Evaluation of antioxidant activity of two imporang memory enhancing medicinal plants Bacoppa monnieri and *Centella asiatica*. *Indian Journal of Phamacology*, 44(1), 114 - 117.

Meliana, Y., Harmami, S. B., & Restu, W. K. (2015). Characterization of nanoencapsulated *Centella asiatica* and *Zingiber officinale* extract using combinaton of maltodextrin and gum Arabic as matrix. *IOP Conference Series: Materials Science and Engineering*, 172, 012065.

Mega, M. S. (2000). The cholinergic deficit in Alzheimer's disease: impact on cognition, behaviour and function. *The International Journal of Neuropsychoparmacology*, 3(7), 3 - 12.

Meyer, B., Ferrigni, N., Putnam, J., Jacobsen, L., Nichols, D., & McLaughlin, J. (1982). Brine Shrimp: A convenient general bioassay for active plant constituents. *Planta Medica*, *45*(05), 31 - 34.

Ministry of Health Malaysia (2009). *Clinical Practice Guidelines: Management of Dementia 2nd Edition.* Malaysia: Ministry of Health Malaysia.

Mogosanu, G. D., Grumezescu, A. M., Bejenaru, C., & Bejenaru, L. E. (2017). Natural products used for food preservation. *Food Preservation*, 2017, 365 - 411.

Moghimipour, E., Salimi, A., & Eftekhari, S. (2013). Design and characterization of microemulsion systems for naproxen. *Advanced Pharmaceutical Bulletin*, *3*(1), 63 - 71.

Mohd Zainol, M. K., Abdul-Hamid, A., Abu Bakar, F., & Pak Dek, S. (2009). Effect of drying methods on the degradation of selected flavonoids in *Centella asiatica*. *International Food Research Journal*, 16, 531 - 537.

Moulik, S. P., & Rakshit, A. K. (2006). Physicochemistry and Applications of Microemulsions. *Journal of Surface Science Tehcnology*, 22, 3 – 4, 159 - 186.

Mourtas, S., Lazar, A. N., Markoutsa, E., Duyckaerts, C., & Antimisiaris, S.G. (2014). Multifunctional nanoliposome with curcumin-lipid derivative and brain targeting functionality with potential applications for Alzheimer disease. *European Journal of Medicinal Chemistry*, 80, 175 - 183.

Nair, V. P. & Hunter, J. M. (2004). Anticholineterases and anticholinergic drugs. *Continuing Education Anaesthesia Critical Care & Pain*, 4(5), 164 - 168.

Najjar, R. (2012). *Microemulsion – An Introduction to properties and applications*. Rijeka: InTech, 3 - 30.

Natrajan, D., Srinivasan, S., Sundar, K., & Ravindran, A. (2015), Formulation of essential oil-loaded chitosan-alginate nanocapsules. *Journal of Food and Drug Analysis*, 23(3), 560 - 568.

Natarajan, S., Shunmugiah, K. P., and Kasi. P. D. (2013). Plant traditionally ssed in age-related Brain disorders (Dementia): An ethanopharmacology survey. *Pharmaceutical Biology*, 51(4), 492 - 523.

Ng, L. T. (1998). Amino acid composition of *Centella asiatica*. *Journal of Tropical Forest Products*, 4(2), 212 - 215.

Nik Norulaini, N. A., Setianto, W. B., Sarker, M. Z., Nawi, A. H., Che Yunus, M. A., Kadir, M. (2009). Effects of supercritical carbon dioxide extraction parameters on virgin coconut oil yield and medium-chain triglyceride content. *Food Chemistry*, 116, 193 - 197.

Nigam, S. K. (2015). What do drug transporters really do? *Nature Reviews. Drug Discovery*, 14(1), 29 - 44.

Nirmala, M. J. & Nagarajan, R. (2017). Recent research trend in fabrication and applications of plant essential oil based nanoemulsion. *Journal of Nanomedicine & Nanotechnology*, 8(2), 1 - 10.

Nour, A. H., Khan, M., Sulaiman, A. Z., Batool, T., Nour, A. H., Khan, M. M., & Kormin, F. (2014). In vitro Anti-Acetyl cholinesterase and antioxidant activity of selected Malaysian plants. *Asian Journal of Pharmaceutical and Clinical Research*, 7(3), 93 - 97.

Oboh, G., Akinyemi, A. J., Omojokun, O. S., & Oyeleye, S. I. (2014). Anticholinesterase and antioxidative properties of aqueous extract of cola acuminate seed in vitro. *International Journal Alzheimer's Disease*, 2014, 1 - 8.

Oda, Y. (1999). Choline acetyltransferase: the structure, distribution and pathologic changes in the central nervouse system. *Pathology International*, 49(11), 921 - 937.

Odhav, B., Beekrum, S., Akula, U. and Baijnath, H. (2007). Preliminary assessment of nutritional value of traditional leafy vegetables in KwaZulu-Natal. *South Africa. Journal of Food Composition and Analysis*, 20, 430 - 435.

Okur, N. Ü., Yavaşoğlu, A., & Karasulu, H. Y. (2014). Preparation and evaluation of microemulsion formulations of naproxen for dermal delivery. *Chemical and Pharmaceutical Bulletin*, *62*(2), 135 - 143.

Ong, H. C. (2008). *Vegetables for Health and Healing.* Kuala Lumpur: Utusan Publications & Distributor Sdn. Bhd.

Orhan, I. E. (2012). *Centella asiatica* (L.) Urban: From traditional medicine to modern medicine with neuroprotective potential. *Evidence-based Complementary and Alternative Medicine*, 2012, 1 - 9.

Orhan, I. E., Atasu, E., Senol, F. S., Ozturk, N., Demirci, B., Das, K., & Sekeroglu, N. (2013). Comparative studies on Turkish and Indian *Centella asiatica* (L.) Urban (gotu kola) samples for their enzyme inhibitory and antioxidant effects and phytochemical characterization. *Industrial Crops and Products*, 47, 316 - 322.

Padmaja, R., Arun, P. C., Prashanth, D., Deepak, M., Amit, A., & Anjana, M. (2002). Brine shrimp lethality bioassay of selected Indian medicinal plants. *Fitoterapia*, 73(6), 508 - 510.

Papich, M. G., & Martinez, M. N. (2015). Applying Biopharmaceutical Classification System (BCS) Criteria to Predict Oral Absorption of Drugs in Dogs: Challenges and Pitfalls. *The American Association of Pharmaceutical Scientists Journal*, 17(4), 948 - 964.

Pardridge, W. M. (2001). Crossing the blood-brain barrier: are we getting it right? *Drug Discovery Today*, 6, 1 - 2.

Parth, S., Surajpal, V., & Plakshi, M. (2016). Global need for novel herbal drug formulations. *International Journal of Pharmacognosy and Phytochemical Research*, 8(9), 1535 - 1544.

Patel, R. B., Patel, M. R., Bhatt, K. K. & Patel, B. G. (2013). Formulation consideration and characterization of microemulsion drug delivery system for transnasal administration of carbamazepine. *Bulletin of Faculty of Pharmacy, Cairo University*, 51, 243 - 253.

Phaniendra, A., Jestadi, D. B., & Periyasamy, L. (2015). Free radicals: properties, sources, targets, and their implication in various diseases. *Indian Journal of Clinical Biochemistry*, 30(1), 11–26.

Pietta, P., Simonetti, P., & Mauri, P. (1998). Antioxidant Activity of Selected Medicinal Plants. *Journal of Agricultural and Food Chemistry*, 46(11), 4487-4490

Pohanka, M. (2014). Inhibitors of acetylcholinesterase and butyrylcholinesterase meet immunity. *International Journal of Molecular Sciences*, 15(6): 9809 - 9825.

Poutan, C. W. (2006). Formulation of poorly water-soluble drugs for oral administration: Physicochemical and physiological issues and the lipid formulation classification system. *European Journal of Pharmaceutical Sciences*, 29, 278 - 287.

Rahman, M. M, Sayeed, M. M. B., Haque, M. A, Hassan, M. M., & Islam S. M. A. (2012). Pytocemical screening, antioxidant, anti-Alzheimer and anti-diabetic activities of *Centella Asiatica*. *Journal Natural Product Plant Resources*, 2(4), 504 - 511.

Rahim, N. S., Lim, S. M., Mani, V., Abdul Majeed, A. B., & Ramasamy, K. (2017). Enhanced memory in Wistar rats by virgin coconut oil is associated with increased antioxidative, cholinergic activities and reduced oxidative stress. *Pharmaceutical Biology*, 55(1), 825 - 832.

Rai, M., Cordell, G. A., Martinez, J. L., Marinoff, M., & Rastrelli, L. (2012). *Medical Plants Biodiversity and Drugs*. CRC Press.

Rao, M. K., Rao, M. S., & Rao, G. S. (2007). Treatment with *Centella asiatica*(Linn) freash leaf extract enhances learning ability and memory retention power in rats. *Neuroscience*, 12(3), 236 - 241.

Rao, S. B., Chetana, M. and Devi, P. U. (2005). *Centella asiatica* treatment during postnatal period enhances learning and memory in mice. *Physiology & Behaviour*, 86, 449 - 457.

Rattanakom, S., & Yusarin, P. (2015). Chemical profiling of *Centella asiatica* under different extraction solvents and its antibacterial activity, antioxidant activity. *Oriental Journal of Chemistry*, 31(4), 2453 - 2459.

Reger M. A., Henderson S. T., Hale C., Cholerton B., Baker L. D., Watson G. S., et al. (2004). Effects of beta-hydroxybutyrate on cognition in memory-impaired adults. *Neurobiology of Aging*, 25, 311 - 314.

Ricci, M., Blasi P., Giovagnoli S., & Rossi, C. (2006). Delivering drugs to the central nervous system: a medicinal chemistry or a pharmaceutical technology issue? *Current Medicinal Chemistry*, 13(15), 1757 - 1775.

Rosenberry T. L. (1975). Acetylcholinesterase. *Advances in Enzymology and Related Areas of Molecular Biology*, 43:103 - 218.

Rukmini, A., Raharjo, S., Hastuti P., & Supriyadi, S. (2012). Formulation and stability of water-in-virgin coconut oil microemulsion using ternary food grade nonionic surfactants. *International Food Research Journal*, 19(1), 259 - 264.

Rungsardthong Ruktanonchai, U., Srinuanchai, W., Saesoo, S., Sramala, I., Puttipipatkhachorn, S., & Soottitantawat, A. (2011). Encapsulation of citral isomers in extracted lemongrass oil with cyclodextrins: molecular modeling and physiochemical characterizations. *Bioscience, Biotechnology and Biochemistry*, 75 (12), 2340 - 2345.

Salsa, T. (1997). Oral controlled release dosage forms: cellulose ether polymers in hydrophilic matrices. *Drug Development and Industrial Pharmacy*, 23(9), 929.

Saoji, S. D., Raut, N. A., Dhore, P. W., Borkar, C. D., Popielarczyk, M. & Dave, V. S. (2015). Preparation and evaluation of phospholipid-based complex of standardized *Centella asiatica* (SCE) for enhanced delivery of phytoconstituents. *The American Association of Pharmaceutical Scientists*, 18(1), 102 - 114.

Saranya, S., Nair, V. A., Prathapan, P., Neethu, A. S., & Kumar, N. S. (2017). Phytochemical analysis of *Centella Asiatica* L. leaf extracts. *International Journal of Advanced Research*, 5 (6), 1828 - 1832.

Sathish, U., Shravani, B., Raghavendra Rao N. G., Srikanth Reddy, M., & Sanjeev, B. (2013). Overview on controlled release dosage form. *International Journal of Pharma Sciences*, 3(4), 258 - 269.

Saxena, G., Singh, S. P., Agrawal, R., & Nath, C. (2008). Effect of donepezil and tacrine on oxidative stress in intracerebral streptozotocin-induced model of dementia in mice. *European Journal Pharmacology*, 581, 283 - 289.

Scheibmeir, H.D., Christensen, K., Whitaker, S.H, Jegaethesan, J., Clancy, R. & Pierce, J.D. (2005). A review of free radicals and antioxidants for critical care nurses. *Intensive and Critical Care Nursing*, 21, 24 - 28.

Shah, G., Shri, R., Panchal, V., Sharma, N., Singh, B., & Mann, A. S. (2011). Scientific basis for the therapeutic use of *Cymbopogon citratus*, stapf (Lemon grass). *Journal of Advanced Pharmaceutical Technology & Research*, *2*(1), 3 - 8.

Shahidi, F., Janita, P. K., & Wanasundara, P. D. (1992). Phenolic antioxidants. *Critical Reveiws in Food Science and Nutrition*, 32(1), 67-103.

Sharma, A. K., Pavlova, S. T., Kim, J., Kim, J., & Mirica, L. M. (2013). The effect of Cu(2+) and Zn(2+) on the Aβ42 peptide aggregation and cellular toxicity. *Metallomics : integrated biometal science*, *5*(11), 1529–1536.

Siegel, G. J., Albers, R. W., Brady, S. T., & Price, D. L. (2006). *Basic Neurochemistry: Molecular, Cellular and Medical Aspects*. 7th Ed. California: Elsevier Academic Press.

Silva, A. E., Barratt, G., Cheron, M., & Egito, E. S. T. (2013). Development of oil-in-water microemulsions for oral delivery of amphotericin B. *International Journal of Pharmaceutics*, 454, 641 - 648.

Silva, C. D. B. D., Guterres, S. S., Weisheimer, V. & Schapoval, E. E. (2008). Antifungal activity of the lemongrass oil and citral against Candida spp. *Brazilian Journal of Infectious Diseases, 12(1),* pp. 63 - 66.

Singh, S., Gautam, A., Sharma, A., & Batra, A. (2010). *Centella asiatica* (L.): A Plant with immense medicinal potential buy threatened. *International Journal of Pharmaceutical Sciences Review and Research*, 4(2), 9 - 17.

Sintov, A. C. (2015). Transdermal delivery of curcumin via microemulsion. *International Journal of Pharmaceutics*, *481*(1), 97 - 103.

Sisinthy, S. P., Lynn Sarah, C. Y., & Rao, N. K. (2014). Optimization of coconut oil based self micro emulsifying drug delivery systems of Olmesartan medoxomil by simplex centroid design. *International Journal of Applied Pharmaceutics*, 8(4), 47 - 52.

Smith, M. W., & Gumbleton, M. (2006). Endocytosis at the blood-brain barrier: from basic understanding to drug delivery strategies. *Journal of Drug Targeting*, 14, 191 - 214.

Sosa, V., Moliné, T., Somoza, R., Paciucci, R., Kondoh, H., & Lleonart, M. E. (2013). Oxidative stress and cancer: An overview. *Ageing Research Reviews*, 12, 376 - 390.

Soumyanath, A., Zhong, Y. P., Henson, E., Wadsworth, T., Bishop, J., Gold, B. G., & Quinn, J. F. (2012). Centella asiatica Extract Improves Behavioral Deficits in a Mouse Model of Alzheimer's Disease: Investigation of a Possible Mechanism of Action. *International journal of Alzheimer's disease*, 2012, 381974.

Sriamornsak, P., Piriyaprasarth, S., Chansiri, G., Promboot, W., Imerb, U., & Sumpoung, D. (2012). Effect of Coconut Oil and Surfactants on Stability of Nanoemulsions. *Advanced Materials Research*, 506,429 - 432.

Stamatovic, S. M., Keep, R. F., & Andjelkovic, A. V. (2008). Brain endothelial cell-cell junctions: How to "open" the blood brain barrier. *Current Neuropharmacology*, 6, 179 - 192.

Subathra, M., Shila, S., Devi, M. A., & Panneerselvam, C. (2005). Emerging role of *Centella asiatica* in improving age-related neurological antioxidant status. *Experimental Gerontology*, 40, 707 - 715.

Subongkot, T., & Ngawhirunpat, T. (2017). Development of a novel microemulsion for oral absorption enhancement of all-trans retinoic acid. *International Journal of Nanomedicine*, 12, 5585 - 5599.

Suganthy, N. & Pandima Devi, K. (2016). In vitro antioxidant and anticholinesterase activities of Rhizophora mucronata, *Pharmaceutical Biology*, 54:1, 118 - 129.

Sultan, R. A., Mahmood, Z. A., Azhar, I., Ul Hassan, M. M., Ahmed, S. (2012). Pharmacognostic and phytochemical investigation of aerial parts of *Centella asiatica* Linn. *International Journal of Phytomedicine*, 4, 125 - 133.

Swaminathan, A., & Jicha, G. A. (2014). Nutrition and prevention of Alzheimer's dementia. *Frontiers in Aging Neuroscience*, 6, 282.

Syed, H. K., & Peh, K. K. (2014). Identification of phases of various oil, surfactant/co-surfactant and water system by ternary phase diagram. *Acta Poloniae Pharmaceutica – Drug Research*, 71(2), 301 - 309.

Tarawneh, R., & Holtzman, D. M. (2012). The clinical problem of symptomatic Alzheimer disease and mild cognitive impairment. *Cold Spring Harbor Perspectives in Medicine*, 2(5), a006148.

Telofski, L. S., Morello, A. P., Mack Correa, M. C., & Stamatas, G. N. (2012). The infant skin barrier: can we preserve, protect, and enhance the barrier?. *Dermatology Research and Practice*, 2012, 1 - 18.

Temple, N.J. (2000). Antioxidants and Disease: More questions than answers. *Nutrition Research*, 20(3), 449 - 459.

Teng, Y., Lian, S., Liu, Q., Liu, Y., Song, Y., He, R., & Zhi, K. (2016). Evolvement behavior of microstructure and H_2O absorption of lignite pyrolysis. *Chinese Journal of Chemical Engineering*, 24(6), 803 - 810.

Tiwari, G., Tiwari, R., Sriwastawa, B., Bhati, L., Pandey, S., Pandey, P., & Bannerjee, S. K. (2012). Drug delivery system: An updated review. *International Journal Pharmaceutical Investigation*, 2(1), 2 - 10.

Torchilin, V. P. (2006). Multifunctional nanocarriers. *Advanced Drug Delivery Reviews*, 58(14), 1532 - 1555.

Tsume, Y., Mudie, D. M., Langguth, P., Amidon, G. E., & Amidon, G. L. (2014). The Biopharmaceutics Classification System: subclasses for in vivo predictive dissolution (IPD) methodology and IVIVC. *European journal of pharmaceutical sciences: official journal of the European Federation for Pharmaceutical Sciences*, 57, 152 - 163.

Urso, M. L. & Clarkson, P. M. (2003). Oxidative stress, exercise and antioxidant supplementation. *Toxicology*, 189, 41 - 54.

Upadhyay, R. K. (2014). (2014). Drug delivery systems, CNS protection, and the blood brain barrier. *BioMed Research International*, 2014, 1 - 37.

Vaishali, A. G., Rupali, N. A., Sagar, A. D. (2016). Analysis of bioactive compounds in leaves extract of *Centella asiatica* by using HRLC-MS & IR techniques. *Journal of Chemical and Pharmaceutical Research*, 8(8), 122 - 125.

Valko, M., Morris, H. & Cronin, M.T.D. (2005). Metals, toxicity and oxidative stress. *Current Medicinal Chemistry*, 12, 1161 - 1208.

Van Hoogevest, P., Liu, X., & Fahr, A. (2011). Drug delivery strategies for poorly water-soluble drugs: the indrustrial perspective. *Expert Opinion on Drug Delivery*, 8(11), 1481 - 1500.

Vasavi, H. S., Arun, A. B., & Rekha, P. D. (2016). Anti-quorum sensing activity of flavonoid rich fraction form *Centella asiatica* L. against Pseudomonas aeruginosa PAO1. *Journal of Microbiology, Immunology and Infection*, 49, 8 - 15.

Vasiliou, V., Vasiliou, K., & Nebert, D. W. (2009). Human ATP-binding cassette (ABC) transporter family. *Human Genomics*, *3*(3), 281 - 290.

Villarino, B. J., Dy, L. M., & Lizada, C. C. (2007). Descriptive sensory evaluation of virgin coconut oil and refined, bleached and deodorized coconut oil. *LWT – Food Science and Technology*, 40, 193 - 199.

Wan Salleh, W. M. N. H., Hashim, N. A., Ahmad, F., & Yen, K. H. (2014). Anticholinesterase And Antityrosinase Activities of ten species from Malaysia. *Advance Pharmaceutical Bulletin*, 4(2), 527 - 531.

Wang, L. H., Wang, C. C., & Kuo, S. C. (2007). Vehicle and enhancer effects on human skin penetration of aminophylline from cream formulations: evaluation in vivo. *Journal of Cosmetic Science*. 58(3), 245 - 254.

Warad, S. B., Kolar, S. S., Kalburgi, V., & Kalburgi, N. B. (2013). Lemongrass essential oil gel as a local drug delivery agent for the treatment of periodontitis. *Ancient Science of Life*, *32*(4), 205 - 211.

Winsor, P. A. (1954). *Solvent Properties of Amphiphilic Compo*unds. Butterworth, London. pp. 40.

Wolburg, H., & Lippoldt, A. (2002). Tight junctions of the blood-brain barrier: developments, composition and regulation. *Vascular Pharmacology*, 38, 323 - 337.

Xi, Y., Frost, R. L., & He, H. (2007). Modification of the surfaces of Wyoming montmorillonite by the cationic surfactants alkyl trumethyl, dialkyl dimethyl, and trialkul methyl ammonium bromides. *Journal of Colloid and Interface Science*, 305(1), 150 - 158.

Yew, H. C., & Misran, M. (2016). Nonionic mixed surfactant stabilized water-in-oil microemulsions for active ingredient in vitro sustained release. *Journal of Surfactants and Detergents*, 16, 49 - 56.

Yiannopoulou, K. G., & Papageorgiou, S. G. (2013). Current and future treatments for Alzheimer's disease. *Therapeutic Advances in Neurological Disorders*, *6*(1), 19 - 33.

Yongsirasawad, K., & Yusarin, P. (2017). Simulated gastrointestinal system study of centella asiatica extract-loaded gelatin nanopartilces on antioxidant and antimicrobial activities. *Journal Applied Sciences Technology*, 10(3), 231 - 238.

Yoshida, M., *et al*., (2005). Antiproliferative constituents from Ubelliferae plant VII. active triterpens and rosmanaric acid from *Centella asiatica*. *Biological & Pharmaceutical Bulletin*, 28(1), 173 - 175.

Yu, L. X., Gatlin, L., & Amidon, G. (2000). *Predicting oral drug absorption in human. Transport processes in pharmaceutical*. New York: Marcel Dekker, 377 - 409.

Zainol, N. A., Voo, S. C., Sarmidi, M. R., & Aziz, R. A., (2008). Profiling of *Centella asiatica* (L.) urban extract. *The Malaysian Journal of Analytical Sciences,* 12(2), 322 - 327.

Zaveri, M., Gajjar, H., Kanaki, N., & Patel, S. (2011). Preparation and evaluation of drug phospholipids complex for increasing transdermal penetration of phyto constituents. *International Journal of Institutional Pharmacy and Life Sciences*, 1(3), 80 - 93.

Zhang, X. F., Liu, Z. G., Shen, W., & Gurunathan, S. (2016). Silver Nanoparticles: Synthesis, Characterization, Properties, Applications, and Therapeutic Approaches. *International Journal of Molecular Sciences*, 17(9), 1 - 34.

Zoheir, N., Lappin, D. F., & Nile, C. J. (2012). Acetylcholine and the alpha 7 nicotinic: a potential therapeutic target for the treatment of periodontal disease?. *Inflammation research: official journal of the European Histamine Research Society… [et al.]*, 61(9), 915 - 926.

Zlokovic, B. V. (2008). The blood-brain barrier in health and chronic neurodegenerative disorders. *Neuron,* 57(2), 178 – 201.

Zucker J., & Zucker, S. (2016). *The Memory Diet*. New Jersey: The Career Press.